T4 DNA Ligase, Cloned

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T4 DNA Ligase is the most versatile and commonly used ligase for DNA cloning. This ATP-dependent enzyme covalently joins 5'-phosphates to 3'-hydroxylated termini at the blunt or compatible cohesive ends of double-stranded DNA fragments produced by restriction enzyme digestion or other enzymatic processes.[1,2] T4 DNA Ligase has no activity on single-stranded nucleic acids. Following a ligation reaction, T4 DNA Ligase may be inactivated by incubation at 65°C for 10 minutes.

EPICENTRE offers T4 DNA Ligase at both standard (2 U/µl) and high (10 U/µl) concentrations. High concentration T4 DNA Ligase is useful for obtaining maximum efficiency in blunt-end ligations. T4 DNA Ligase is supplied with a 10X Reaction Buffer and a 25 mM ATP Solution.

Applications

- Ligation of blunt or cohesive-ended DNA fragments (Figure 1).
- Repair of nicks in double-stranded nucleic acids.[3]

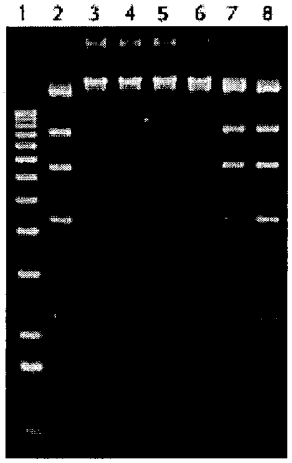

Figure 1. Ligation activity of EPICENTRE's T4 DNA Ligase. T4 DNA Ligase at 10 U/µl was diluted sequentially in 10-fold increments. One microliter of each dilution of T4 DNA Ligase was incubated with Hind III-cut lambda DNA at 16°C in 1X Reaction Buffer containing ATP. Lane 1, kb ladder; Lane 2, no enzyme; Lane 3, 1 U/µl; Lane 4, 0.1 U/µl; Lane 5, 0.01 U/µl; Lane 6, 0.001 U/µl; Lane 7, 0.0001 U/µl; Lane 8, 0.00001 U/µl. Note that full enzyme activity is seen down to one one-thousandth unit; enzyme activity is still apparent even at one ten-thousandth unit.

Unit Definition: One Weiss unit of T4 DNA Ligase converts 1 nmole of ^{32}P from pyrophosphate into Norit-adsorbable material in 2 minutes at 37°C in 33 mM Tris-acetate (pH 7.8), 66 mM potassium acetate, 10 mM magnesium acetate, 0.5 mM DTT, and 1 mM ATP.[4] One Weiss unit equals approximately 100 cohesive-end ligation units.

Storage Buffer: 50% glycerol containing 50 mM Tris-HCl (pH 7.5), 0.1 M NaCl, 0.1 mM EDTA, 0.1% Triton X-100, and 1 mM DTT

T4 DNA Ligase 10X Reaction Buffer: 330 mM Tris-acetate (pH 7.8), 660 mM potassium acetate, 100 mM magnesium acetate, and 5 mM DTT. The Reaction Buffer does not contain ATP, which must be added to the reaction to a final concentration of 0.5 - 1.0 mM. A 25 mM solution of ATP is included.

Quality Control: T4 DNA Ligase is functionally tested in cloning assays and is free of detectable contaminating DNA exo- and endonuclease and RNase activities.

References

1. Helfman, D.M. *et al.* (1987) *Meth. Enzymol.* **152**, 349.
2. Wu, R. *et al.* (1987) *Meth. Enzymol.* **152**, 343.
3. Sambrook, J. *et al.* (1989) in: *Molecular Cloning: A Laboratory Manual* (2nd ed.), Cold Spring Harbor Laboratory Press, Ne York.
4. Weiss, B. *et al.* (1968) *J. Biol. Chem.* **243**, 4543.

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Catalog No.	Concentration	Size
T4 DNA Ligase, Cloned		
L0805H	2 U/µl	500 U
L0810H	2 U/µl	1,000 U
L0820H	2 U/µl	2,000 U
LH805H	10 U/µl	500 U
LH810H	10 U/µl	1,000 U
LH820H	10 U/µl	2,000 U

Includes 10X Reaction Buffer and a separate 25 mM ATP Solution.

T4 DNA Ligase is also available in bulk. Please inquire.

You may wish to consider the following related products:

Ampligase® Thermostable DNA Ligase
Colony Fast-Screen™ Kit (PCR Screen) NEW
Colony Fast-Screen™ Kit (Size Screen)
Fast-Link™ DNA Ligation and Screening Kit
Fast-Link™ DNA Ligation Kit
HK™ Thermolabile Phosphatase
Mung Bean Nuclease
T4 DNA Polymerase
T4 Polynucleotide Kinase, Cloned
T4 RNA Ligase

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18	1	6117679.pn. and ligas$4	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2004/08/16 15:43
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22	11	stemmer.in. and flap	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2004/08/16 16:18
23	5085	overlap$4 near2 flap	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2004/08/16 16:16
24	6	(overlap$4 near2 flap) and (shuffl$4)	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2004/08/16 16:17
25	14	(overlap$4 near2 flap) and (flap near5 cleav$5)	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2004/08/16 16:18
26	123	stemmer.in. and overlap$5	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2004/08/16 16:19
27	92	(stemmer.in. and overlap$5) and willem.in.	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2004/08/16 16:20
28	90	((stemmer.in. and overlap$5) and willem.in.) and (cleav$5 digest$5)	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2004/08/16 16:21

29	4	((stemmer.in. and overlap$5) and willem.in.) and ((cleav$5 digest$5) near5 (single adj1 strand (ss)))	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2004/08/16 16:41
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31	105	(flap adj1 endonuclease) and clon$4	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2004/08/16 16:43
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